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Biology, host specificity tests, and risk assessment of the sawfly *Heteroperreyia hubrichi*, a potential biological control agent of *Schinus terebinthifolius* in Hawaii

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Abstract. *Heteroperreyia hubrichi* Malaise (Hymenoptera: Pergidae), a foliage feeding sawfly of *Schinus terebinthifolius* Raddi (Sapindales: Anacardiaceae), was studied to assess its suitability as a classical biological control agent of this invasive weed in Hawaii. No-choice host-specificity tests were conducted in Hawaiian quarantine on 20 plant species in 10 families. Besides the target weed, adult females oviposited on four test species. Females accepted the Hawaiian native *Rhus sandwicensis* A. Gray (Sapindales: Anacardiaceae) as an oviposition host equally as well as the target species. The other three species received significantly fewer eggs. Neonate larvae transferred onto test plants successfully developed to pupae on *S. terebinthifolius* (70% survival) and *R. sandwicensis* (1% survival). All other 18 test plant species failed to support larval development. A risk analysis was conducted to quantify the acceptability of non-target species as host plants for *H. hubrichi* on the basis of the insect's performance at various stages in its life cycle. Risk of damage to all plant species tested was insignificant except for *R. sandwicensis*. Risk to this native plant relative to *S. terebinthifolius* was estimated at 1%. Currently this level of risk is too high to request introduction of this insect into the Hawaiian environment. Detailed impact studies in the native range of *S. terebinthifolius* are needed to identify the potential benefit that this insect offers. Also, field studies in South America with potted *R. sandwicensis* would give a more reliable analysis of the risk this native Hawaiian plant would face from natural populations of *H. hubrichi*.

Key words: Anacardiaceae, Brazilian peppertree, Christmasberry, classical biological control, *Heteroperreyia hubrichi*, host specificity, non-target impacts, *Rhus sandwicensis*, risk assessment, *Schinus terebinthifolius*

Introduction

Schinus terebinthifolius Raddi (Sapindales: Anacardiaceae), locally known as Christmasberry in Hawaii, or Brazilian peppertree in Florida, is an introduced perennial plant that has become established throughout the Hawaiian Islands (Yoshioka and Markin, 1991). This species is native to Argentina, Brazil, and Paraguay (Barkley, 1944, 1957) and was brought to Hawaii as an ornamental before 1900 (Neal, 1965).

In Hawaii, *S. terebinthifolius* has become an aggressive, rapidly spreading weed that displaces native vegetation (Bennett et al., 1990; Cuddihy and Stone, 1990). As early as the 1940's, *S. terebinthifolius* was recognized as an important invader of dry slopes on Oahu (Egler, 1942). The plant is recognized as a noxious weed by the Hawaii Department of Agriculture (Morton, 1978). Conservation organizations consider Christmasberry a high priority target in Hawaii because it is already widespread and has great potential to increase its range even further (Randall, 1993). The U.S. Fish and Wildlife Service (1998) identified *S. terebinthifolius* as one of the most significant non-indigenous species currently threatening federally listed threatened and endangered native plants throughout the Hawaiian islands. Attributes of the plant that contribute to its invasiveness include a large number of fruits produced per female plant, an effective mechanism of dispersal by birds (Panetta and McKee, 1997), tolerance to shade (Ewel, 1978), fire (Doren et al., 1991), and drought (Nilsen and Muller, 1980), and an apparent allelochemical effect on neighboring plants (Medal et al., 1999).

Classical biological control against Christmasberry was initiated in Hawaii in mid-1900s (Yoshioka and Markin, 1991). Surveys were conducted in South America (primarily Brazil) for potential biological control agents (Krauss, 1962, 1963). Three insect species native to Brazil were released into Hawaii: a seed-feeding beetle, *Lithraeus* (=*Bruchus*) *atronotatus* Pic (Coleoptera: Bruchidae), in 1960 (Davis, 1961; Krauss, 1963); a leaf-rolling moth, *Episimus utilis* Zimmerman (Lepidoptera: Tortricidae), in 1954–1956 (Beardsley, 1959; Davis, 1959; Krauss, 1963); and a stem-galling moth, *Crasimorpha infuscata* Hodges (Lepidoptera: Gelechiidae), in 1961–1962 (Davis and Krauss, 1962; Krauss, 1963). The first two species became established but are reported to cause only minor damage (Clausen, 1978; Yoshioka and Markin, 1991; Hight, in preparation). A seed-feeding wasp, *Megastigmus transvaalensis* (Hussey) (Hymenoptera: Torymidae), accidentally introduced from South Africa, has been found attacking seeds of Christmasberry in Hawaii since early 1970s (Beardsley, 1971; Yoshioka and Markin, 1991). Infestation rates of this wasp on Christmasberry seeds have continued to increase (Hight, unpublished data).

Recent classical biological control efforts against *S. terebinthifolius* have been focused in Florida since the late 1980s. This plant is listed as one of Florida's worst noxious weeds (Bennett and Habeck, 1991; Habeck, 1995). Exploratory surveys for natural enemies in Brazil identified at least 200 species of arthropods associated with *S. terebinthifolius* (Bennett et al., 1990; Bennett and Habeck, 1991; Medal et al., 1999). Several insects were selected as biological control candidates for further study in Florida (Medal et al., 1999). Host specificity studies were conducted on the Brazilian schinus sawfly *Heteroperreyia hubrichi* Malaise (Hymenoptera: Pergidae) in Brazil and a Florida quarantine facility (Medal et al., 1999). Larval development and female oviposition tests of *H. hubrichi* were conducted on 36 plant species in 15 families. The insect was determined to be host specific to *S. terebinthifolius* and a request for releasing this insect into the Florida environment is currently under a risk assessment by Animal and Plant Health Inspection Service, USDA (Medal et al., 1999).

Capitalizing on biological studies and host specificity tests conducted in Brazil and Florida, a biological control project was initiated to evaluate the potential of *H. hubrichi* as a control agent of *S. terebinthifolius* in Hawaii. Since host specificity is essential for a biological control agent, we present an investigation of the host range of *H. hubrichi* in Hawaiian quarantine. We assess the risk to non-target plants if this insect were to be released into the Hawaiian environment. This paper also reports on several aspects of the biology of this species not reported elsewhere.

Materials and methods

Twenty plant species underwent host specificity testing in the Hawaii Volcanoes National Park Quarantine facility from March 1999 through May 2000. The selected plants belonged to one of three groupings: taxonomically associated plants, habitat associated native plants, and habitat associated agricultural plants (Table 1). Plant relatedness is based on the phylogenetic system of Cronquist (1981). The taxonomic treatment followed is that of Wagner et al. (1990). The order Sapindales has 15 families and four of these families (Anacardiaceae, Rutaceae, Sapindaceae, and Zygophyllaceae) have native Hawaiian members (as well as introduced species into Hawaii). The single, native, Hawaiian species of Zygophyllaceae, *Tribulus cistoides* L., was not tested because it occurs only in coastal habitats below 50 m elevation (Wagner et al., 1990). Of the remaining 11 families, only Meliaceae has members introduced into Hawaii. Plants that make up the second group are native plants that occur in the same habitat and are therefore likely to be exposed to any introduced biological control agent. The second group are not

as closely related to *S. terebinthifolius*, although members in three families (Araliaceae, Myrtaceae, and Fabaceae) are in the same subclass (Rosidae). The third group contains two important, woody, crops that are found associated with *S. terebinthifolius* habitat. These two species are in the same subclass as *S. terebinthifolius*.

Insect material

Two shipments of *H. hubrichi* were imported from Brazil into the Hawaii Volcanoes National Park Quarantine facility. The first shipment was received 19 November 1998 and consisted of 236 neonate larvae which eclosed from four egg masses and 192 late instar larvae. The second shipment arrived 22 March 1999 and contained 101 late instar larvae. Individuals of both shipments were collected in southern Brazil in the vicinity of Curitiba, Province of Paraná. Quarantine host specificity tests were conducted from subsequent generations reared in captivity. All reported measures of central tendencies are means $\pm$ standard errors.

Both male and female adults can fly, although the male is a stronger flyer. Neither the male or female adult *H. hubrichi* feed. However, both sexes were observed drinking from small water droplets.

Adult oviposition tests

No-choice oviposition tests were conducted on cut shoots for each of the 20 test plant species. An approximately 20 cm stem section of a test plant was fitted through an open-ended florist tube. The tube was placed through a hole in the bottom of a 960 ml plastic container and a hole in the lid of a 480 ml plastic container. The 480 ml container was filled with water and about 7 cm of the test plant stem was submersed in this water. The terminal 13 cm section of the stem (with 2 to 4 leaves) was enclosed in the 960 ml 'oviposition test arena'.

Newly emerged individual male and female *H. hubrichi* were placed together in 15 mm $\times$ 100 mm petri dishes for mating. The number of copulations and length of each copulation were recorded. Matings involving males that mated with more than one female were evaluated to insure their success and eventual egg viability.

A mated female *H. hubrichi* was placed on the test plant and the top of the oviposition arena secured with a fine mesh cloth screening. If a female oviposited on the test plant, she remained inside the arena with her eggs. If a female did not oviposit on the test plant within 48 to 60 hr, she was removed and placed in a new oviposition arena with a stem of *S. terebinthifolius* to evaluate her fecundity. Number of eggs laid, time that it took for initiating

Table 1. Host specificity tests on cut shoots for the Brazilian schinus-sawfly *Heteroperreyia hubrichi* in Hawaiian quarantine

Plant family	Plant species[1]	Adult Oviposition		Larval Development		Avg. larval survival (%)		
		Reps.	No. eggs oviposited (mean ± SE)	Reps. : Total No. individuals	Larval feeding[2]	3 days	7 days	Final
A. Taxonomically Associated Plants								
Anacardiaceae	*Schinus terebinthifolius* Raddi	35	115 ± 23.0	87 : 1032	+ + +	94	88	70
	**Rhus sandwicensis* A. Gray	11	118 ± 20.7	24 : 401	+ +	72	24	1
	Mangifera indica L.	6	0	6 : 120	(+)	97	92	0
Sapindaceae	**Dodonaea viscosa* Jacq.	24	5 ± 21.2	6 : 90	–	2	0	0
	**Sapindus saponaria* L.	4	0	6 : 90	–	8	0	0
	Litchi chinensis Sonn.	16	34 ± 49.8	6 : 90	(+)	32	0	0
	Euphoria longan Lam.	12	23 ± 36.6	10 : 159	(+)	53	17	0
	Alectryon subcinereum Gaertn.	5	0	6 : 90	–	8	8	0
	Nephelium mutabile L.	6	0	8 : 120	–	12	0	0
Rutaceae	**Melicope hawaiensis* (Wawra) T. Hartley & B. Stone	6	0	6 : 90	–	37	0	0
	Citrus sinensis (L.) Osbeck	0	0	7 : 105	–	57	0	0

Table 1. Continued

Plant family	Plant species[1]	Adult Oviposition		Larval Development		Avg. larval survival (%)		
		Reps.	No. eggs oviposited (mean ± SE)	Reps. : Total No. individuals	Larval feeding[2]	3 days	7 days	Final
B. Habitat Associated Native Plants								
Araliaceae	**Reynoldsia sandwicensis* A. Gray	5	0	6 : 91	–	41	0	0
Myrtaceae	**Metrosideros polymorpha* Gaud.	4	0	7 : 104	–	62	0	0
Fabaceae	**Acacia koa* A. Gray	4	0	12 : 180	–	2	0	0
	**Sophora chrysophylla* (Salisb.) Seem.	4	0	12 : 180	–	17	6	0
Myoporaceae	**Myoporum sandwicense* A. Gray	4	0	6 : 90	–	39	0	0
Convolvulaceae	**Ipomoea indica* (J. Burm.) Merr.	6	0	6 : 90	–	37	0	0
Dicksoniaceae	**Cibotium glaucum* (Sm.) Hook. & Arnott	4	0	6 : 90	–	16	0	0
C. Habitat Associated Agricultural Plants								
Proteaceae	*Macadamia integrifolia* Maiden & Betche	4	0	6 : 90	–	29	0	0
Rubiaceae	*Coffea arabica* L.	4	0	6 : 90	–	4	0	0

[1] * = Species native to Hawaii.

[2] + + + indicates normal larval feeding; + + indicates moderate larval feeding; + indicates slight larval feeding; (+) indicates occasional nibbling by larvae; and – indicates that no larval feeding occurred.

egg laying, and longevity of female was recorded. For each plant species, tests were replicated at least six times. Oviposition arenas were set up in the quarantine under ambient conditions: January monthly, average, temperatures are 20 °C high, 7 °C low, with 13 h dark: 11 h light; July monthly, average, temperatures are 22 °C high: 12 °C low, with 11 h dark: 13 h light; and relative humidity fluctuating daily between 55–100% year round.

Oviposition tests were again conducted on potted plants of six test species because of oviposition activity and/or larval development on cut shoots of those six test species. A well established potted plant of a test species was placed in a 50 cm × 50 cm × 45 cm wooden cage inside quarantine. A mated female was placed on the test plant and remained on that plant until she died. The number of eggs laid, time that it took for egg laying to be initiated, longevity of female, and viability of eggs was recorded. Each plant species was replicated at least six times.

No-choice larval development tests

All test plants were evaluated as to their ability to support larval development under no-choice conditions. Neonate larvae, less than two hours old, were transferred to small cut shoots of the test plant stuck into moistened florist foam filled vials and reared in 480 ml plastic containers. We insured that neonate larvae had not fed before being transferred, and that they were offered fresh, newly expanding, terminal leaves of each test plant. Also, we fed maturing larvae cut stems with terminal leaves that were not stored more than two days. Preliminary rearing tests had identified that larvae through the 2nd instar stage were sensitive to older leaves (Hight, unpublished data). Since larvae feed gregariously, 15 larvae were transferred into each container with a fine tip brush. Each test plant was replicated at least six times. For each family of larvae used in the tests, 2–3 replicates of 10–15 larvae were reared on *S. terebinthifolius* to insure the vitality of each egg mass. Rearing containers were placed on a bench in quarantine in a completely randomized design. Larvae were reared in quarantine under ambient conditions (see above). Containers were cleaned, larvae were fed, and mortality was assessed on the third day after transfer and then every fourth day. Containers were evaluated every day after larvae became sixth instars.

Larval development tests were also conducted on potted plants of six test species because of oviposition activity and/or larval development on cut shoots. Well established potted plants of each test species was placed inside quarantine. Depending on the size of the plant, the plant was either contained in a 50 cm × 50 cm × 45 cm wooden cage or under a netted bag. Each plant of *S. terebinthifolius* and *Rhus sandwicensis* A. Gray (Sapindales: Anacardiaceae) had an egg mass of *H. hubrichi* naturally oviposited on the stem. Each

potted plant of three Sapindaceae test species [*Dodonaea viscosa* Jacq., *Litchi chinensis* Sonn., and *Euphoria longan* (Lour.) Steud.] had an egg mass tied onto a stem from a successful oviposition on *S. terebinthifolius*. The number of larvae that successfully developed on each test plant was recorded. Each plant species was replicated at least six times.

Choice larval tests

Separate choice tests were conducted in six cages to see if neonate larvae that had initiated feeding would remain on *R. sandwicensis* or move onto *S. terebinthifolius*. A potted plant of *R. sandwicensis* which contained an egg mass of *H. hubrichi* was placed in a 50 cm × 50 cm × 45 cm wooden cage inside quarantine. Thirty-six to 48 hrs after the eggs hatched and larvae had initiated feeding on *R. sandwicensis*, a potted plant of *S. terebinthifolius* was placed in the cage so the leaves of the two plant species were touching. Movement and survival of the larvae were recorded.

Risk assessment

To quantify potential risks to non-target plant species, Wan and Harris (1997) developed a risk analysis scoring system. Risk was determined by measuring the insect's performance on each test plant species at different stages in the host selection process, as a proportion of the performance on the target plant species. In this study relative risk was based on four performance measures (Table 2): (1) adult preference, (2) oviposition preference, (3) larval development, and (4) larval survival. Adult preference and oviposition preference were determined by the proportion of adult females that accepted the test plant for oviposition and the number of eggs oviposited (Table 2). Larval development and larval survival were determined by the time required for larvae to complete development from egg to eclosion and the proportion of eggs that survived to pupation (Table 2).

The risk of *H. hubrichi* utilizing a non-target plant was calculated as the product of $R_1 \times R_2 \times R_3 \times R_4$, where R is the performance measure for the insect on the test plant relative to that on *S. terebinthifolius* (Table 3). In the risk analysis (Table 3), larval development time (R_3) was converted to the inverse proportion of larval development time relative to development time on *S. terebinthifolius*. Performance measures were estimated from experiments conducted on cut shoots, not potted plants. For purposes of calculation, zero values (complete rejection) were taken to be 0.001.

Table 2. Performance of *Heteroperreyia hubrichi* on various host plant test species in Hawaiian quarantine

Component of Host Usage for Risk Assessment	Performance Measure	Host Plant Test Species				
		S. terebinthi-folius	*R. sandwi-censis*	*D. viscosa*	*L. chinensis*	*E. longan*
Establishment	1. proportion of females that oviposit	1.000	1.000	0.083	0.438	0.417
Establishment	2. mean number of eggs oviposited	115	118	5	34	23
Establishment	3. mean development time of larvae (days)	39	47	0	0	0
Damage	4. proportion of eggs that survive to pupae	0.697	0.008	0	0	0

Table 3. Risk analysis of *Heteroperreyia hubrichi* performance measures on various host plant test species in Hawaiian quarantine

Plant Species	Performance Measure[1]				Risk
	R_1	R_2	R_3	R_4	
S. terebinthifolius	1.000	1.000	1.000	1.000	1.000
R. sandwicensis	1.000	1.026	0.830	0.012	0.010
D. viscosa	0.038	0.043	0.001	0.001	3.6×10^{-9}
L. chinensis	0.438	0.296	0.001	0.001	1.3×10^{-7}
E. longan	0.417	0.200	0.001	0.001	8.3×10^{-8}

[1]Performance measure estimates are proportional to *S. terebinthifolius.*
R_1 = proportion of females that oviposit; R_2 = mean number of eggs oviposited; R_3 = inverse proportion of mean development time of larvae; R_4 = proportion of eggs that survive to pupae.

Results

Insect biology

The sex ratio of both Brazilian shipments combined was 1.5 female : 1 male. Throughout this study, the sex ratio for each of the four generations reared in quarantine ranged from 2.5 to 3.0 female : 1 male.

The adults of *H. hubrichi* are generally black in color with yellow legs. A female and male *H. hubrichi* mate on the surface of soil or plants. Females do not need to mate for oviposition to occur but unmated females produce only males. Each female oviposits her eggs in a single mass just into the surface of non-woody stems. Eggs in a mass are arranged in rows and the female 'guards' her eggs until she dies, just before the eggs hatch. Females lived 8 $\pm$ 2.7 d (n = 102) and males lived 7 $\pm$ 2.8 d (n = 64) in our experimental oviposition arenas. All eggs in a single mass hatched at the same time. Eggs hatched in 13 $\pm$ 0.8 d (n = 38 masses). Neonate larvae feed gregariously on both surfaces of young leaflets at the tip of shoots. As they grow they move as a group onto new leaflets and larger leaves until the third to fourth instar when they disperse throughout the plant and feed individually. Larvae are green with red spots and black legs. After reaching the seventh instar, the larva moves into soil and pupates. For 913 larval sawflies successfully reared in the quarantine facility on *S. terebinthifolius* between March and July, egg hatch to pupation took 23–85 d (39 $\pm$ 10.2 d). The pupal stage lasted two months for most individuals (81%). Other pupae lasted 3 months (14%) and the latest emergence occurred within 7 months (< 1%).

Females appeared to mate successfully only once. Initial copulations that formed fertile eggs between a female and male lasted 18 $\pm$ 4.0 min (n = 60). Subsequent matings were 'discouraged' by the female and lasted only a few seconds (if at all) whether the same or a different male attempted copulation. Mated females were placed on test plants within 5 min after copulation ceased. After females were place on test plants they began oviposition on *S. terebinthifolius* cut stems in 45 $\pm$ 37.2 min (n = 21) but took only 18 $\pm$ 26.0 min (n = 33) to initiate oviposition on cut stems of *R. sandwicensis*. Upon contact with the test plant, most females immediately walked up and down the stem as though evaluating the plant as an acceptable oviposition host.

Adult oviposition tests

Female *H. hubrichi* oviposited on cut shoots on five of the 20 different test plant species (Table 1). All females that were placed on *S. terebinthifolius* and *R. sandwicensis* oviposited on their test plant. Of the other three test

plant species that received eggs, less than half of the females successfully oviposited on their test plant (Table 2; Performance Measure 1). However, all non-ovipositing females tested on the 18 plant species successfully oviposited once they were moved onto *S. terebinthifolius* after the 48–60 hr test period. This indicated that the females were capable of ovipositing on the test plant but rejected that plant as an oviposition host.

Mean number of eggs oviposited by females on each test plant species is presented in Table 1. Even though *R. sandwicensis* received on average more eggs than *S. terebinthifolius*, the difference was not significant when evaluated with a *t*-test at the significance level of 0.05 ($t_{(43)} = 1.762$). Oviposition tests on the three Sapindaceae species that received eggs was highly variable with most replicates receiving no eggs. For those plants receiving eggs the average was fairly high: *Dodonaea viscosa* Jacq. – 57 eggs (8% of females oviposited); *Litchi chinensis* Sonn. – 78 eggs (32% of females oviposited); and *Euphoria longan* (Lour.) Steud. – 56 eggs (42% of females oviposited).

Oviposition was more restrictive on potted plants than cut shoots. Only three of the five species of potted test plants received oviposition from mated *H. hubrichi* females (*S. terebinthifolius*, *R. sandwicensis*, and *E. longan*). All mated females oviposited on potted plants of *S. terebinthifolius* and *R. sandwicensis* while only 25% of females oviposited on potted *E. longan*. Females did not oviposit on potted *D. viscosa* or *L. chinensis*, even though oviposition did occur on cut shoots of *D. viscosa* and *L. chinensis*.

No-choice larval transfer tests

Neonate larvae successfully developed on cut shoots of only two test plant species, *S. terebinthifolius* and *R. sandwicensis*. Larvae on most of the other test plant species were dead within seven days (Table 1). Although cut shoots of two additional plant species supported some larval development for more than two weeks, *Mangifera indica* L. (Sapindales: Anacardiaceae) (23 d) and *E. longan* (19 d), no larvae survived to pupation.

Successful larval development on the five potted plant species was similar to development on cut shoots. Larvae developed only on potted *S. terebinthifolius* and *R. sandwicensis*. Larval development slightly improved on both plant species when larvae developed on potted plants. Survival of larvae from egg to pupa on *S. terebinthifolius* increased from 70% (n = 1032) to 81% (n = 1329) and on *R. sandwicensis* from 1% (n = 401) to 7% (n = 2669). Larval development time was reduced on *S. terebinthifolius* from 39 ± 10.2 d (n = 913) to 38 ± 6.0 d (n = 734) and on *R. sandwicensis* from 47 ± 0.0 d (n = 3) to 43 ± 7.2 d (n = 175).

Choice larval tests

In three cages larvae appeared too weak to move off of *R. sandwicensis* onto *S. terebinthifolius* and no larvae survived. Many larvae in the other three cages moved onto *S. terebinthifolius* and an average of 41% (± 12.1%) survived and pupated. Larvae that remained on *R. sandwicensis* did not survive. Later instar larvae periodically moved back onto *R. sandwicensis* leaves for brief periods but did not feed.

Risk assessment

The 'risk', or relative host suitability, of the test plant species is shown in Table 3. Risk estimates are calculated only for the five plant species which received eggs on cut stems from ovipositing females. Scores for all four non-target plants were lower than the risk to *S. terebinthifolius*, measured at 1.0. All other 15 test plant species were unacceptable hosts for both oviposition and larval development and are not at risk by the release of *H. hubrichi* into Hawaii.

Discussion

Field observations in Brazil and laboratory feeding tests in Florida indicated that *H. hubrichi* was highly host specific and safe to release into the Florida environment (Medal et al., 1999). Additional host specificity studies in quarantine on primarily native Hawaiian plants confirmed a highly limited host range for *H. hubrichi*. Tests at all locations showed that *S. terebinthifolius* was the preferred, if not only, host plant of *H. hubrichi*. However, the potential host range in Hawaii appears to be slightly broader than that identified in Florida and Brazil. Tests in Florida evaluated two North American species of sumac *(R. copallina* and *R. michauxii*) and found them unsuitable for *H. hubrichi* oviposition and incapable of supporting larval development (Medal et al., 1999; J. Cuda, personal communication). Hawaiian tests indicated that the Hawaiian sumac (*R. sandwicensis*) did support larval development and was highly attractive to the female for oviposition. Chemicals still present in ancestral, continental species that deter herbivorous insects may have been lost over time in the Hawaiian sumac; although no data exist to confirm this hypothesis. Of the five varieties of *S. terebinthifolius* recognized in South America (Barkley, 1944), *H. hubrichi* prefers the most pubescent variety (M. Vitorino, unpublished data). The dense pubescent nature of *R. sandwicensis* may stimulate female oviposition regardless of the quality of the plant for larval development. Both *S. terebinthifolius* and *R. sandwicensis*

were comparable in their acceptance by ovipositing females as measured by proportion of females that oviposited on the test plant and the number of eggs that a female laid. But *R. sandwicensis* was a dramatically poor host for *H. hubrichi* larvae in both performance characteristics of larval survival and development time.

A risk assessment was conducted to identify the potential non-target effect that native *R. sandwicensis* might be exposed to because of the introduction and release of *H. hubrichi* into Hawaii. Assessing the risk to non-target plants requires analysis of two stages: the probability of the biological control agent establishing on the non-target and the damage inflicted on the non-target (Withers, 1999; Withers et al., 1999). To arrive at realistic probabilities of risk, both physiological and behavioral processes must be estimated (McEvoy, 1996). The probability of establishment on a test plant was quantified as the product of crucial stages in the sawflies sequence to locate and accept the host, i.e. oviposition by the female and larval development time. Damage to the test plant was estimated as the probability of larvae surviving from egg to pupa in no-choice tests.

Non-target, risk assessment was evaluated for the five test plant species that experienced any probability of establishment and/or damage from *H. hubrichi* in the host specificity tests. Three plant species had extremely low probabilities of risk (Table 3). In fact, since all three of these plants completely failed to support larval development it may be argued that their risk from *H. hubrichi* is zero. The life cycle of *H. hubrichi* would be interrupted if the insects were to colonize any one of these plants and a population of *H. hubrichi* would fail to establish. A measurable risk of non-target impact from *H. hubrichi* was recorded for *R. sandwicensis*, although the probability was low (approximately 1%).

Introduction of *H. hubrichi* into Hawaii will not be requested at this time because of the estimated non-target risk to *R. sandwicensis*. However, additional information is being sought which may reverse this decision. Field experiments in Brazil with potted *R. sandwicensis* are being proposed to evaluate the risk of this non-target plant under more natural settings. Host range of herbivorous insects has been considered wider under laboratory-based tests than open-field tests (Cullen, 1990; Briese, 1999). In addition, the risk inherent in introducing a biological control agent may be outweighed by its benefit. Therefore, detailed, ecological, impact studies are needed in Brazil to evaluate the effect *H. hubrichi* has on *S. terebinthifolius* fitness. Neither of these types of tests are currently funded.

Additional surveys for phytophagous insects of *S. terebinthifolius* need to be conducted in northern Argentina, the most likely center of origin of this species (Barkley, 1944). Virtually all previous South American explorations

by Hawaii (Krauss, 1962, 1963) and Florida (Bennett et al., 1990; Bennett and Habeck, 1991) have taken place in southern Brazil. Although this work has identified several promising biological control candidates, additional surveys may be more successful in Argentina. For example, on a 10 day survey in January 2000 of *S. terebinthifolius* natural enemies in the state of Missiones, Argentina, two species of stem boring Cerambycidae and a bark girdling Buprestidae were collected in *S. terebinthifolius* (S. Hight, unpublished data). Identifications of these insect species are pending. No stem boring or bark girdling insects were identified from earlier Brazilian surveys.

Predicting the risk that an introduced biological control agent poses to native plants is difficult but imminently important. Although practitioners of classical biological control of weeds strive to limit non-target attacks, several cases have been documented that reveal impact to non-target hosts (Louda et al., 1997; Pemberton, 2000). Supplementing host specificity tests with ecological studies on non-target plants will be time consuming but will likely decrease non-target impacts. Ecological studies will improve predictions about field interactions with and the population consequences for a native plant that was acceptable to but not preferred by a biological control agent (Arnett and Louda, 2002). In addition, incorporating a risk analysis such as Wan and Harris (1997) allows a quantitative estimation of non-target plant usage, aiding biological control decisions made by regulators, land managers, and scientists. Olckers (2000) was the first to utilize this analysis to successfully present information to regulators on a biological control program against *Solanum mauritianum* Scopoli (Solanales: Solanaceae) in South Africa.

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